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Proof of concept in a tissue co-culture system

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Mark Tommerdahl
Robert Dennis

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Department of Biomedical Engineering, University of North Carolina at Chapel Hill, NC 27599-7575

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14. ABSTRACT
The objective of the proposed work was to demonstrate that bi-directional communication via synthetic electrode arrays can be established between engineered tissues in cell culture (muscle and nerve) and that this communication can be exploited to control a remotely located robotic actuator. Specific applications of this technology will include peripheral nerve interfaces for the neurally-based feedback control of human prosthetic devices, and the control of remote actuators and robotic agents via a direct peripheral nerve interface. Progress has been steady but slower than anticipated. Our progress is described in detail in the Summary of results.

15. SUBJECT TERMS
nerve-muscle interface; bi-directional communication; neural feedback

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Final Report: Nerve Muscle Interface for Prosthetic Feedback Control
Proof of concept in a tissue co-culture system

ARO STIR: 52297-LS-II

Mark Tommerdahl, Ph.D.
Robert G. Dennis, Ph.D.

(1) Foreword

The objective of the proposed work was to demonstrate that bi-directional communication via synthetic electrode arrays can be established between engineered tissues in cell culture (muscle and nerve) and that this communication can be exploited to control a remotely located robotic actuator. Specific applications of this technology will include peripheral nerve interfaces for the neurally-based feedback control of human prosthetic devices, and the control of remote actuators and robotic agents via a direct peripheral nerve interface. Progress has been steady but slower than anticipated. Our progress is described in detail in the Summary of results.

(2) Table of contents

(3) List of Appendixes, Illustrations and Tables

List of Appendixes and Tables – not applicable
List of Illustrations

(4) Statement of the problem studied.

The objective of the proposed work was to demonstrate that bi-directional communication via synthetic electrode arrays can be established between engineered tissues in cell culture (muscle and nerve) and that this communication can be exploited to control a remotely located robotic actuator. Specific applications of this technology will include peripheral nerve interfaces for the neurally-based feedback control of human prosthetic devices, and the control of remote actuators and robotic agents via a direct peripheral nerve interface.

TECHNICAL APPROACH

Established cell lines (commercially available, not directly derived from laboratory animals) will be used to create self-organizing 3-dimensional tissue constructs in culture. Our laboratory has considerable experience with the development of 3-D functional self-organizing tissues and tissue interfaces. We will build upon our recent success with the development of functional tissues and tissue interfaces in culture to create innervated "target" tissues in a controlled cell culture environment. These tissue constructs will be assessed for function based on their typical tissue-level function: muscle organs contract to generate controllable force, work and power, nerve axons depolarize and transmit action potentials, and a functional nerve-muscle interface will allow transduction of an axonal signal to the muscle tissue, resulting in contraction.

(5) Summary of the most important results.

Executive Summary

Progress has been steady but slower than anticipated. Our progress is mapped to each milestone in the proposal, as detailed below:

MILESTONES (as stated in the approved Proposal)
1- Demonstration of muscle-nerve co-culture system with axonal sprouting
2- Demonstration of synaptogenesis in culture using cell lines
3- Demonstration of electrode system in culture for bi-directional neural interface
4- Neural imaging functional correlation with electrode mapping
5- Interface with external robotic agent
6- Technical summary and recommendations for further work

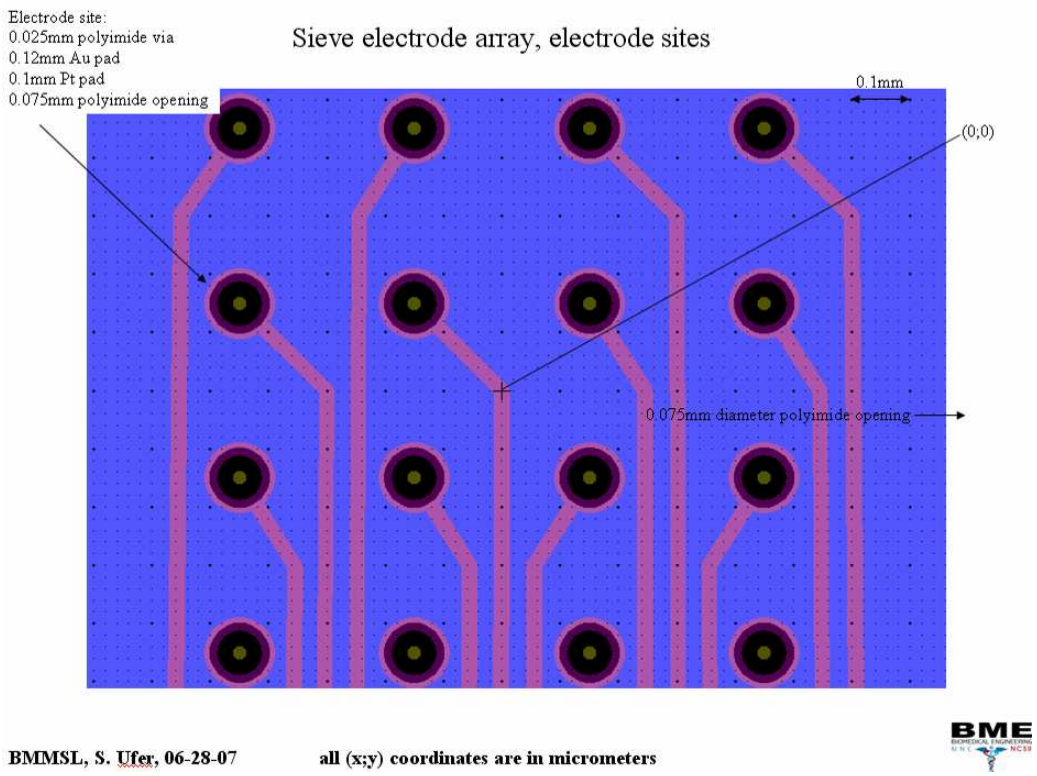
Milestone accomplishment status to date (9-30-2007):

1- Muscle-nerve co-culture system with axonal sprouting: cell lines were selected and grown individually, we are still developing the co-culture media formulation to support the co-culture.
2- Synaptogenesis in culture using cell lines: this depends upon completion of milestone 1 (above)
3- Electrode system in culture for bi-directional neural interface: The electrode arrays have been designed and the first run has been fabricated (11 electrode arrays). They are currently under functional test. The supporting electronics has been designed and prototypes have been tested. A final circuit board is being designed at this time. The interface connectors have been purchased and are being assembled onto the electrode arrays.
4- Neural imaging functional correlation with electrode mapping: Neural imaging methods were developed, but correlation of data depends upon completion of the milestones above.
5- Interface with external robotic agent: This has been designed (actually, three alternatives have been designed) by the BME senior design class of 2007-08, and they will be ready for use by 5 December 2007.

Specific Technical Accomplishments:

<u>Electrode Array and Interface Electronics:</u>
Electrode array was designed, built, and is currently under test for patch-to-patch impedance. The schematic and technical specifications for the 4 x 4 electrode array is shown in the images below:

The electrode array is a 4 x 4 square matrix of instrumented holes as shown. The center-to-center pitch of the electrode array is 300 µm. Each electrode has platinum/gold electrode with a 25 µm open via to allow axonal penetration. In co-culture, the nerve cells will be placed on one side of the electrode array and the engineered muscle will be placed on the other.



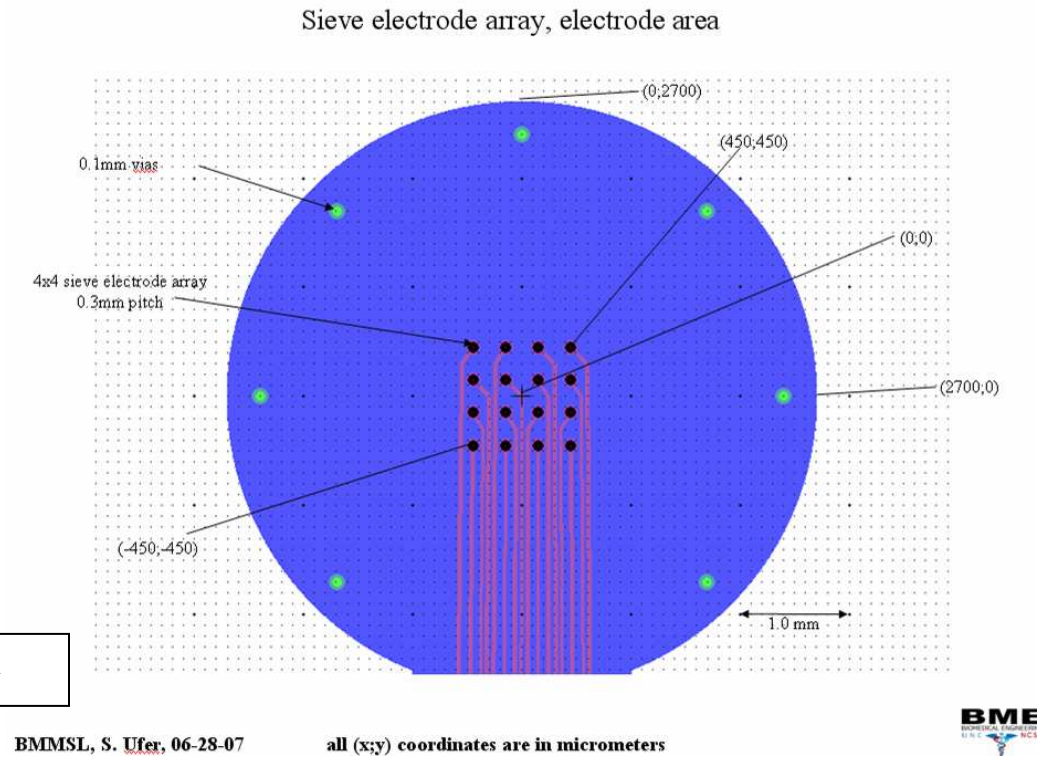
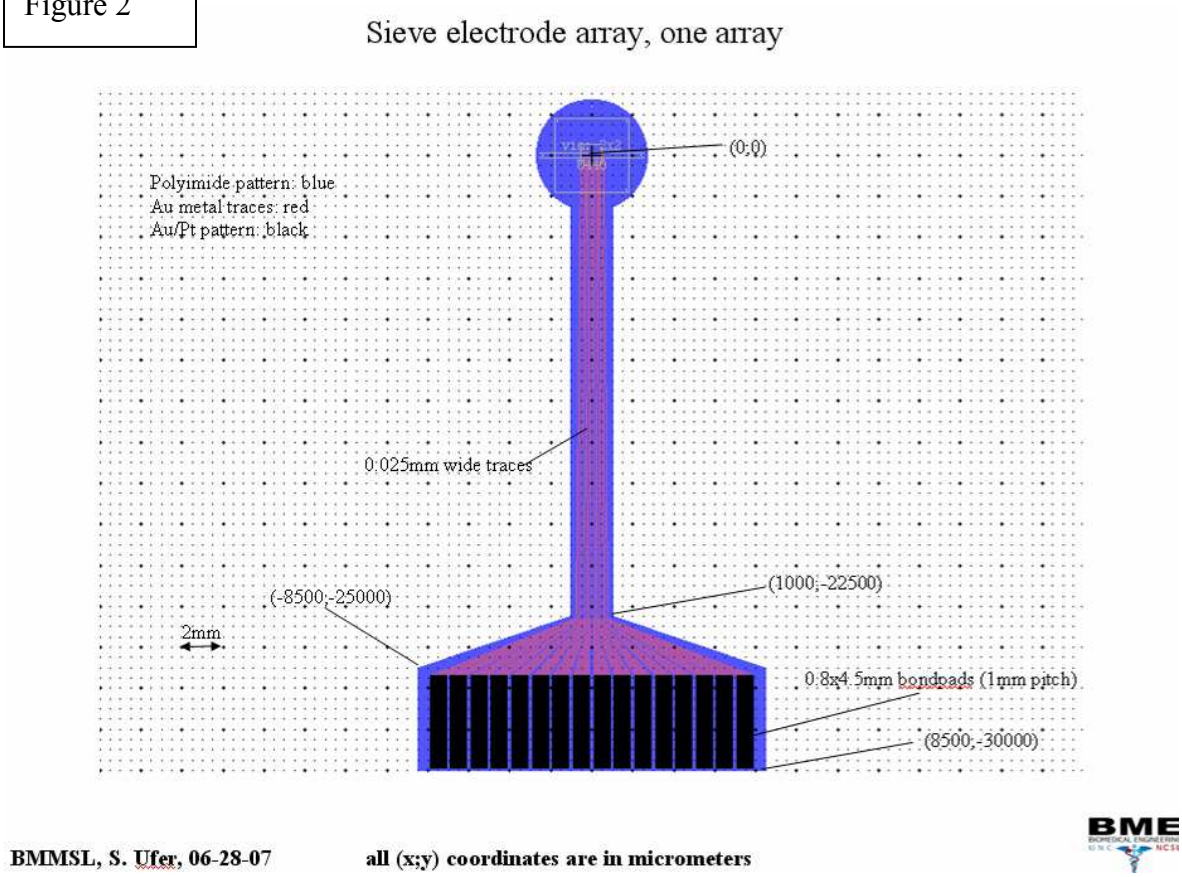


Figure 1

The 4 x 4 electrode array has an isolation plane of non-conductive polyimide substrate that extends 2mm in radius around the active array as shown in the image above. This isolation plane serves two functions: (1) to prevent axon growth around the electrode array, thereby bypassing the active electrode sites, and (2) to allow attachment of the electrode to tissues using suture (7 holes near the periphery, each 0.1mm in diameter, have been provided to facilitate suturing).

Figure 2



Thin polyimide sieve electrode array, 4 x 4 active sites with Au/Pt sputtering is shown above with the traces extending to the bondpads for the interface connector. Each electrode is 75 um diameter with 25 um vias for axonal projection from the nerve cell culture to the muscle tissue culture on the opposite side of the electrode array. Pitch between electrode sites is 300 microns. Total electrode system thickness is 25 um. The completed electrode array is shown below, photographed using a stereo dissecting microscope:

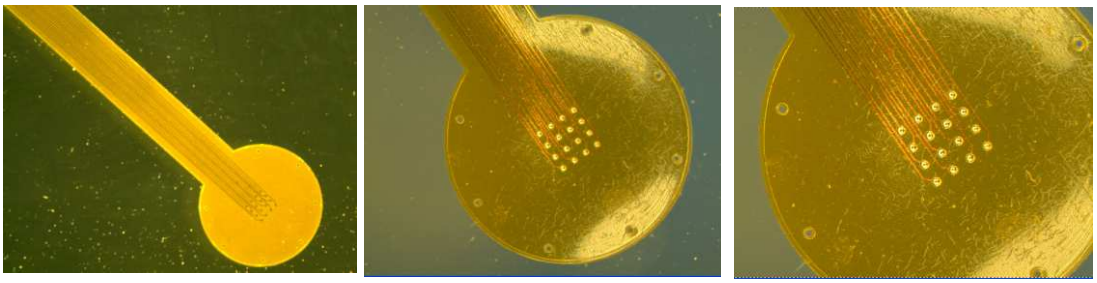


Figure 3

The first prototype schematic of the electrical interface circuit is shown below. The circuit will feature independent analog input/output channels for each of the 16 active electrode sites on the 4 x 4 electrode array. Each channel has independent optical isolation and a FET pre-amplifier. Each channel is essentially "quad-state", allowing the channel to be set to (1) high-impedance state (inactive), (2) ground state, (3) low-output impedance state (stimulation mode), or (4) input state through an optically-isolated high input impedance pre-amplifier. This circuit will interface with a commercially available analog and digital data acquisition system (B&B electronics USB-DAQ), and a custom-built stimulus train pulse generator.

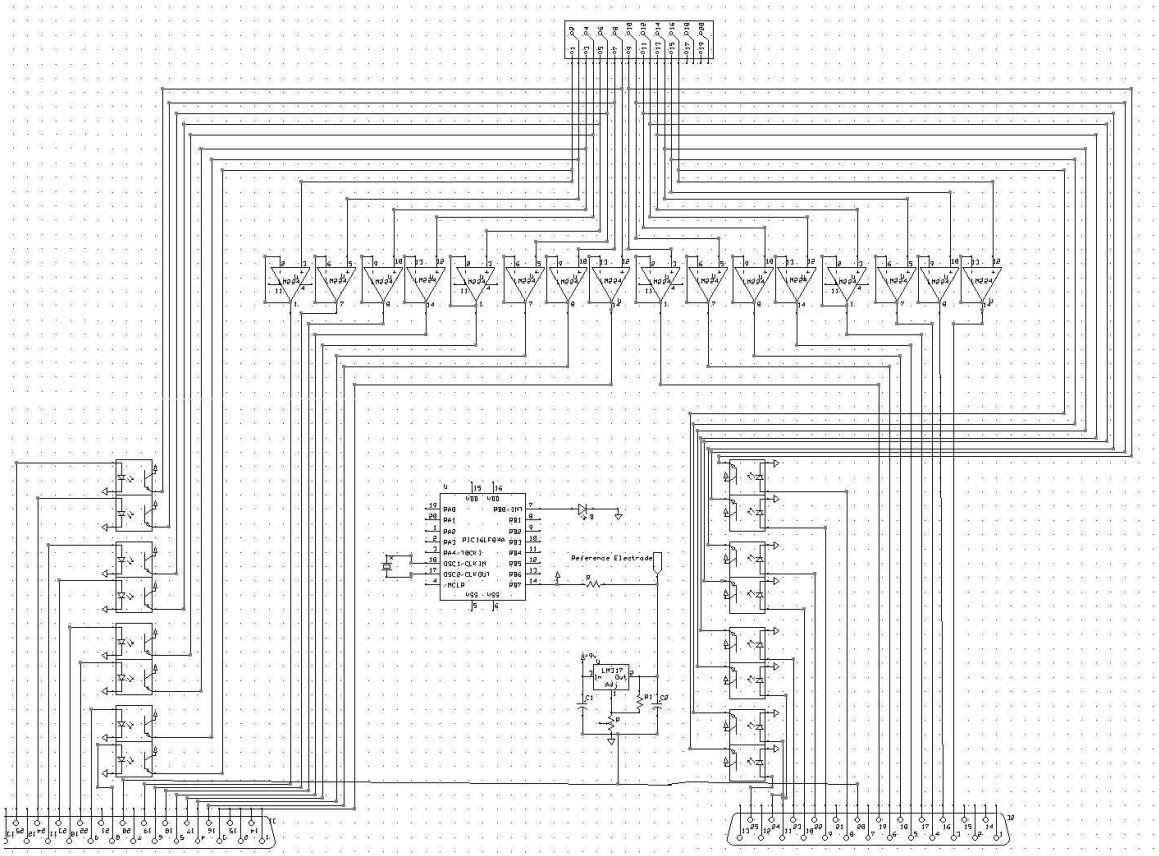


Figure 4

Cell culture work:
We have utilized commercially available cell lines in this research. These include ATCC cell lines: NG108-15 (neural cells), C2C12 (a murine-derived fusion –capable skeletal muscle cell line), and 10T1/2 (a fibroblast lineage that enhances differentiation of the other cells and provides extracellular matrix (ECM) proteins to help mechanically stabilize the tissue constructs in culture.

We have grown out the three cell lines and have as a result discovered a contamination problem in the cell culture facility. This problem, traced to a single individual, has been corrected. A second run of cell cultures is underway at the time of this report, which includes initially the culturing of the muscle constructs in vitro using the C2C12 and 10T1/2 cell lines. This process requires approximately 4 weeks, and the constructs remain stable in culture for several months thereafter. When the muscle constructs have matured (end of October), co-culture experiments with the NG108-15 cells sill resume.

Robotic Agent:
Three teams of senior BME design students at UNC are each developing wirelessly controlled robotic agents that respond to biopotential signal control inputs. One of these will be selected for use in the final technical stages of this project. All three projects are well ahead of schedule and we anticipate no difficulties whatsoever with this aspect of the project.

(6) Listing of all Publications acknowledging this grant.

(a) Papers published in peer-reviewed journals: None

(b) Papers published in non-peer-reviewed journals or in conference proceedings: None

(c) Papers presented at meetings, but not published in conference proceedings

None

(d) Manuscripts submitted, but not published

None

(e) Technical reports submitted to ARO

None

(7) List of all participating scientific personnel showing any advanced degrees earned by them while employed on the project

none

(*8) Report of Inventions (by title only)*

none

(9) Bibliography

(10) Appendices - none